Atty. Dkt. No. 069748-0304

AMENDMENTS TO THE SPECIFICATION

Please replace the first full paragraph on page 1, with the following rewritten paragraph:

This application is a Divisional of Application Serial No. 09/388,089, filed August 31, 1999, ~~currently allowed~~ now USP No. 6,693,186, and claims priority benefits of provisional U.S. application No. 60/098,685, filed Sep. 1, 1998, the entire disclosure of which is incorporated by reference herein.

Please replace Table 3.1 beginning at line 10 on page 7, with the following rewritten Table:

3.1. DEFINITIONS AND ABBREVIATIONS

anti-NMASP	= a polyclonal or monoclonal antibody or antiserum that binds to a NMASP polypeptide or NMASP-derived polypeptide
ATCC®	= American Type Culture Collection
blebs	= naturally occurring outer membrane vesicles of *Neisseria meningitides*
antigenic	= capable of binding specifically to antibody or T cell receptors and provoking an immune response
immunogenic	= capable of provoking a protective cellular or humoral immune response
kD	= kilodaltons
N.	= *Neisseria*
NMASP	= a non-cytosolic polypeptide of a *Neisseria meningitidis*, or any strain or cultivar thereof, having a molecular weight of about 40 kD to 55 kD;

NMASP-derived polypeptide	= fragment of the NMASP polypeptide; variant of wild-containing one or more amino acid deletions, insertions or substitutions; or chimeric protein comprising a heterologous polypeptide fused to a C-terminal or N-terminal or internal segment or a whole or a portion of the NMASP polypeptide
OG	= n-octyl β-D-glucopyranoside or octyl glucoside
PBS	= phosphate buffered saline
PAG	= polyacrylamide gel
polypeptide	= a peptide or protein of any length, preferably one having eight or more amino acid residues
SDS	= sodium dodecylsulfate
SDS-PAGE	= sodium dodecylsulfate polyacrylamide gel electrophoresis

Please replace the last full paragraph on page 9, with the following rewritten paragraph:

~~FIG. 2~~ FIGS. 2A and 2B: NMASP protein expressed from TOP10(pNmAH145), uninduced (SDS: lane 2, Western blot: lane 4) and IPTG induced (SDS: lane 3, Western blot: lane 5). A monoclonal anti-(His).sub.5 antibody conjugated to HRP (QiaGen®) was used to identify the protein and visualization of the antibody reactive pattern was achieved on Hyperfilm™ using the Amersham ECL chemiluminescence system. Lane 1 shows Novex® MultiMark molecular weight markers of myosin (250 kD), phosphorylase B (148 kD), glutamic dehydrogenase (62 kD), carbonic anhydrase (42 kD), myoglobin-blue (30 kD), myoglobin-red (22 kD), and lysozyme (17 kD).

Please replace the third full paragraph on page 10, with the following rewritten paragraph:

In particular embodiments, the NMASP polypeptide is that obtainable from any of Neisseria meningitidis, including, but not limited to types A-L and W. Preferred are N.m. Type A, Type B, Type C and Type W. Strains from any of these organisms may be obtained worldwide from any biologicals depository, particularly strains of N.m. Type A: ATCC® (American Type Culture Collection)13077, ATCC® (American Type Culture Collection)53417; Type B ATCC® (American Type Culture Collection)13090, ATCC® (American Type Culture Collection)13091, ATCC® (American Type Culture Collection)13092, ATCC® (American Type Culture Collection) 13093, ATCC® (American Type Culture Collection) 13094, ATCC® (American Type Culture Collection) 13096, ATCC® (American Type Culture Collection) 13098, ATCC® (American Type Culture Collection) 13100, ATCC® (American Type Culture Collection)23247, ATCC® (American Type Culture Collection)23249, ATCC® (American Type Culture Collection)23250, ATCC® (American Type Culture Collection)23251, ATCC® (American Type Culture Collection)23253, ATCC® (American Type Culture Collection)23254, ATCC® (American Type Culture Collection)23255, ATCC® (American Type Culture Collection)23583, ATCC® (American Type Culture Collection)33086, ATCC® (American Type Culture Collection)53044, ATCC® (American Type Culture Collection)53415, ATCC® (American Type Culture Collection)53418; Type C ATCC® (American Type Culture Collection)13102, ATCC® (American Type Culture Collection)13103, ATCC® (American Type Culture Collection)13105, ATCC® (American Type Culture Collection)13106, ATCC® (American Type Culture Collection)132107, ATCC® (American Type Culture Collection)13108, ATCC® (American Type Culture Collection)13109, ATCC® (American Type Culture Collection)13110, ATCC® (American Type Culture Collection)13111, ATCC® (American Type Culture Collection)13112, ATCC® (American Type Culture Collection)23252, ATCC® (American Type Culture Collection)23248, ATCC® (American Type Culture Collection)31275, ATCC® (American Type Culture Collection)53414, ATCC® (American Type Culture Collection)53416, ATCC® (American Type Culture Collection)53900; and Type 29-E ATCC® (American Type Culture Collection)35558.

Please replace the second full paragraph on page 17, with the following rewritten paragraph:

The NMASP polypeptide of the invention may be isolated from a protein extract including a whole cell extract, of any Neisseria meningitidis, including, but not limited to, types A-L and W. Preferred are Nm. Type A, Type B, Type C and Type W. Strains from any of these organisms may be obtained worldwide from any biologicals depository, particularly strains of N.m. Type A: ATCC® (American Type Culture Collection)13077, ATCC® (American Type Culture Collection)53417; Type B ATCC® (American Type Culture Collection)13090, ATCC® (American Type Culture Collection)13091, ATCC® (American Type Culture Collection)13092, ATCC® (American Type Culture Collection)13093, ATCC® (American Type Culture Collection)13094, ATCC® (American Type Culture Collection)13096, ATCC® (American Type Culture Collection) 13098, ATCC® (American Type Culture Collection) 13100, ATCC® (American Type Culture Collection)23247, ATCC® (American Type Culture Collection)23249, ATCC® (American Type Culture Collection)23250, ATCC® (American Type Culture Collection)23251, ATCC® (American Type Culture Collection)23253, ATCC® (American Type Culture Collection)23254, ATCC® (American Type Culture Collection)23255, ATCC® (American Type Culture Collection)23583, ATCC® (American Type Culture Collection)33086, ATCC® (American Type Culture Collection)53044, ATCC® (American Type Culture Collection)53415, ATCC® (American Type Culture Collection)53418; Type C ATCC® (American Type Culture Collection)13102, ATCC® (American Type Culture Collection)13103, ATCC® (American Type Culture Collection)13105, ATCC® (American Type Culture Collection)13106, ATCC® (American Type Culture Collection)132107, ATCC® (American Type Culture Collection)13108, ATCC® (American Type Culture Collection)13109, ATCC® (American Type Culture Collection)13110, ATCC® (American Type Culture Collection)13111, ATCC® (American Type Culture Collection)13112, ATCC® (American Type Culture Collection)23252, ATCC® (American Type Culture Collection)23248, ATCC® (American Type Culture Collection)31275, ATCC® (American Type Culture Collection)53414, ATCC® (American Type Culture Collection)53416, ATCC® (American Type Culture Collection)53900; and Type 29-E ATCC® (American Type Culture Collection)35558. Another source of the NMASP polypeptide is a protein preparation from a gene expression system expressing a

sequence encoding NMASP polypeptide or NMASP-derived polypeptides (see Section 5.7., infra).

Please replace the first full paragraph on page 18, with the following rewritten paragraph:

Another method of purifying NMASP polypeptide is by affinity chromatography using anti-NMASP antibodies, (see Section 5.5). Preferably, monoclonal anti-NMASP antibodies are used. The antibodies are covalently linked to agarose gels activated by cyanogen bromide or succinimide esters (Affi-Gel®, BioRad, Inc.) or by other methods known to those skilled in the art. The protein extract is loaded on the top of the gel as described above. The contact is for a period of time and under standard reaction conditions sufficient for NMASP polypeptide to bind to the antibody. Preferably, the solid support is a material used in a chromatographic column. NMASP polypeptide is then removed from the antibody, thereby permitting the recovery NMASP polypeptide in isolated, or preferably, purified form.

Please replace the first full paragraph on page 20, with the following rewritten paragraph:

In an embodiment, the NMASP polypeptide is separated from other outer membrane or periplasmic proteins present in the extracts of Neisseria meningitidis cells or blebs using SDS-PAGE (see Section 5.3. above) and the gel slice containing NMASP polypeptide is used as an immunogen and injected into a rabbit to produce antisera containing polyclonal NMASP antibodies. The same immunogen can be used to immunize mice for the production of hybridoma lines that produce monoclonal anti-NMASP antibodies. In particular embodiments, the immunogen is a PAG slice containing isolated or purified NMASP from any Neisseria meningitidis, including, but not limited to, types A-L and W. Preferred are N.m. Type A, Type B, Type C and Type W. Particularly preferred are the strains of N.m. Type A: ATCC® (American Type Culture Collection)13077, ATCC® (American Type Culture Collection)53417; Type B ATCC® (American Type Culture Collection)13090, ATCC® (American Type Culture Collection)13091, ATCC® (American Type Culture Collection)13092, ATCC® (American Type Culture Collection)13093, ATCC® (American

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Please replace the paragraph bridging pages 20 and 21, with the following rewritten paragraph:

In yet another embodiment, for the production of antibodies that specifically bind one or more epitopes of the native NMASP polypeptide, intact Neisseria meningitidis cells or blebs prepared therefrom are used as immunogen. The cells or blebs may be fixed with agents such as formaldehyde or glutaraldehyde before immunization. See Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988, Chapter 15. It is preferred that such anti-whole cell antibodies be monoclonal antibodies. Hybridoma lines producing the desired monoclonal antibodies can be identified by using purified NMASP polypeptide as the screening ligand. The immunogen for inducing these antibodies are whole cells, blebs, extracts or lysates of any Neisseria meningitidis, including, but not limited to, types A-L and W. Preferred are N.m. Type A, Type B, Type C and Type W. Particularly preferred are strains of N.m.Type A: ATCC® (American Type Culture Collection)13077, ATCC® (American Type Culture Collection)53417; Type B ATCC® (American Type Culture Collection)13090, ATCC® (American Type Culture Collection)13091, ATCC® (American Type Culture Collection)13092, ATCC® (American Type Culture Collection)13093, ATCC® (American Type Culture Collection)13094, ATCC® (American Type Culture Collection)13096, ATCC® (American Type Culture Collection)13098, ATCC® (American Type Culture Collection)13100, ATCC® (American Type Culture Collection)23247, ATCC® (American Type Culture Collection)23249, ATCC® (American Type Culture Collection)23250, ATCC® (American Type Culture Collection)23251, ATCC® (American Type Culture Collection)23253, ATCC® (American Type Culture Collection)23254, ATCC® (American Type Culture Collection)23255, ATCC® (American Type Culture Collection)23583, ATCC® (American Type Culture Collection)33086, ATCC® (American Type Culture Collection)53044, ATCC® (American Type Culture Collection)53415, ATCC® (American Type Culture Collection)53418; Type C ATCC® (American Type Culture Collection)13102, ATCC® (American Type Culture Collection)13103, ATCC® (American Type Culture Collection)13105, ATCC® (American Type Culture Collection)13106, ATCC® (American Type Culture Collection)132107, ATCC® (American Type Culture Collection)13108, ATCC® (American Type Culture Collection)13109, ATCC® (American Type Culture Collection)13110, ATCC® (American

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Please replace the first full paragraph on page 26, with the following rewritten paragraph:

By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40° C. in a solution containing 35% formamide, 5xSSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll™, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll™, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20X10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40° C., and then washed for 1.5 h at 55° C. in a solution containing 2XSSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60° C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68° C. and re-exposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

Please replace the second full paragraph on page 26, with the following rewritten paragraph:

In another specific embodiment, a nucleic acid which is hybridizable to a nucleic acid encoding NMASP polypeptide or an NMASP-derived polypeptide under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65° C. in buffer composed of 6XSSC, 50 mM Tris-HCl (pH

7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll™, 0.02% BSA, and 500 μ/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65° C. in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20X10.sup.6 cpm of .sup.32P-labeled probe. Washing of filters is done at 37° C. for 1 h in a solution containing 2XSSC, 0.01% PVP, 0.01% Ficoll™, and 0.01% BSA. This is followed by a wash in 0.1XSSC at 50° C. for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

Please replace the second full paragraph on page 28, with the following rewritten paragraph:

In another aspect, the amino acid sequence may be used as a guide for synthesis of oligonucleotide mixtures which in turn can be used to screen for NMASP polypeptide coding sequences in Neisseria meningitidis genomic libraries and PCR amplification products. Preferably the DNA used as the source of the NMASP polypeptide coding sequence, for both genomic libraries and PCR amplification, is prepared from cells of any Neisseria meningitidis, including, but not limited to, types A-L and W. Preferred are N.m. Type A, Type B, Type C and Type W. Strains from any of these organisms may be obtained worldwide from any biologicals depository, particularly strains of Nm.Type A: ATCC® (American Type Culture Collection) 13077, ATCC® (American Type Culture Collection) 53417; Type B ATCC® (American Type Culture Collection) 13090, ATCC® (American Type Culture Collection) 13091, ATCC® (American Type Culture Collection) 13092, ATCC® (American Type Culture Collection) 13093, ATCC® (American Type Culture Collection) 13094, ATCC® (American Type Culture Collection) 13096, ATCC® (American Type Culture Collection) 13098, ATCC® (American Type Culture Collection) 13100, ATCC® (American Type Culture Collection) 23247, ATCC® (American Type Culture Collection) 23249, ATCC® (American Type Culture Collection) 23250, ATCC® (American Type Culture Collection) 23251, ATCC® (American Type Culture Collection) 23253, ATCC® (American Type Culture Collection) 23254, ATCC® (American Type Culture Collection) 23255, ATCC® (American Type Culture Collection) 23583, ATCC® (American Type Culture Collection) 33086, ATCC® (American Type Culture Collection)

53044, ATCC® (American Type Culture Collection) 53415, ATCC® (American Type Culture Collection) 53418; Type C ATCC® (American Type Culture Collection) 13102, ATCC® (American Type Culture Collection) 13103, ATCC® (American Type Culture Collection) 13105, ATCC® (American Type Culture Collection) 13106, ATCC® (American Type Culture Collection) 132107, ATCC® (American Type Culture Collection) 13108, ATCC® (American Type Culture Collection) 13109, ATCC® (American Type Culture Collection) 13110, ATCC® (American Type Culture Collection) 13111, ATCC® (American Type Culture Collection) 13112, ATCC® (American Type Culture Collection) 23252, ATCC® (American Type Culture Collection) 23248, ATCC® (American Type Culture Collection) 31275, ATCC® (American Type Culture Collection) 53414, ATCC® (American Type Culture Collection) 53416, ATCC® (American Type Culture Collection) 53900; and Type 29-E ATCC® (American Type Culture Collection) 35558.

Please replace the paragraph bridging pages 41 and 42, with the following rewritten paragraph:

N. meningitidis ATCC® (American Type Culture Collection) 13090 are grown on gonococcal agar (GC/agar base, Difco; supplemental with 1% Iso Vitale X™, BBL) or chocolate agar plates for 24-48 hours at 37° C. in 5% CO_2. Cells are removed by scraping the colonies from the agar surface using a polystyrene inoculating loop. Cells are then solubilized by suspending 30 µg of cells in 150 µl of PAGE sample buffer (360 mM Tris buffer [pH 8.8], containing 2-mercaptoethanol, 4% sodium dodecylsulfate and 20% glycerol), and incubating the suspension at 100° C. for 5 minutes. The solubilized cells are resolved on 12% polyacrylamide gels as per Laemmli and the separated proteins were electrophoretically transferred to PVDF membranes at 100 V for 1.5 hours as previously described (Thebaine et al. 1979, Proc. Natl. Acad. Sci. USA 35 76:4350-4354). The PVDF membranes are then pretreated with 25 ml of Dulbecco's phosphate buffered saline containing 0.5% sodium casein, 0.5% bovine serum albumin and 1% goat serum. All subsequent incubations are carried out using this pretreatment buffer.

Please replace the first full paragraph on page 42, with the following rewritten paragraph:

PVDF membranes are incubated with 25 ml of a 1:500 dilution of preimmune rabbit serum or serum from a rabbit immunized with NMASP or Hin47 polypeptide (as described above) for 1 hour at room temperature or monoclonal antibodies to NMASP or to Hin47 (described above). PVDF membranes are then washed twice with wash buffer (20 mM Tris buffer (pH 7.5.) containing 150 mM sodium chloride and 0.05% Tween-20®). PVDF membranes are incubated with 25 ml of a 1:5000 dilution of peroxidase-labeled goat anti-rabbit (or anti-mouse for monoclonals) IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa.) for 30 minutes at room temperature. PVDF membranes are then washed 4 times with wash buffer, and are developed with 3,3'diaminobenzidine tetrahydrochloride and urea peroxide as supplied by Sigma Chemical Co. (St. Louis, Mo. catalog number D-4418) for 4 minutes each.

Please replace the paragraph bridging pages 43 and 44, with the following rewritten paragraph:

The E. coli DegP (HtrA) amino acid sequence available from GeneBank™ was employed as a BLAST (TBLASTN) subject query to search the partially completed, crude, and unassembled publicly available genomic sequence databases for N. meningitidis sero-group A (Sanger Center, UK) to identify linear amino acid sequences that might share some similarity to the DegP protein. No predicted amino acid sequences from these Neisseria databases showed more than ~36% sequence identity to the E. coli DegP protein sequence. [% identity determined using TBLASTN program (Altschul et al., 1990, J Molec. Biol. 215:403-10; Altschul et al., 1997, Nuc. Acids Res. 25:3389-3402) with data entered using FASTA format; expect 10 filter default; description 100, alignment as described www.ncbi.nlm.nih.gov.] Candidate NMASP amino acid sequences from the N. meningitidis A database were localized within specific genomic DNA sequence "contigs", and putative open reading frames encoding these NMASP sequences were derived. Putative ORFs capable of encoding proteins of ~40-55 kD, the average size of most DegP-like serine proteases, were then selected and further analyzed for the presence and appropriate relative spacing of semi-conserved catalytic residues (H, D, S) thought to be required for serine protease activity. A single putative open reading frame from the N. meningitidis A database was identified which met these criteria. This putative NMASP ORFs were then compared to each other using a CLUSTAL pairwise

analysis and found to be ~96% identical at the primary amino acid level. These putative ORFs were then used to individually search the partially completed N. meningitidis B genomic database (TIGR, USA) for similar putative NMASP amino acid sequences using the TBLASTN algorithm. These analyses demonstrated that N. meningitidis B strain, like the N. meningitidis A, also contains a putative NMASP ORF that is highly conserved (~97%) compared to those identified in N. meningitidis A.

Please replace the paragraph bridging pages 44 and 45, with the following rewritten paragraph:

N. meningitidis was streaked on gonococcal agar base (GC agar, Difco) containing 1.0% Iso Vitale X™ (BBL) and grown at 35-37° C. in 5% CO_2 for ~24-48 hours. To prepare confluent "lawns" of cells for DNA isolation, three or four single colonies were picked from the "overnight" seed plate and used to inoculate fresh GC plates which were again grown overnight at 35-37° C. in 5% CO_2. Cells were collected from the surface of the agar plates by gentle rinsing using trypticase soy broth (TSB) containing 10% glycerol and then stored at -20° C. When needed, cells were thawed at room temperature and bacteria collected by centrifugation in a Sorval SS34 rotor at ~2000Xg for 15 minutes at room temperature. The supernatant was removed and the cell pellet suspended in ~5.0 ml of sterile water. An equal volume of lysis buffer (200 mM NaCl, 20 mM EDTA, 40 mM Tris-HCl pH8.0, 0.5% (w/v) SDS, 0.5% (v/v) 2-mercaptoethanol, and 250 ug/ml of proteinase K) was added and the cells suspended by gentle agitation and trituration. The cell suspension was then incubated ~12 hours at 50° C. to lyse the bacteria and liberate chromosomal DNA.

Please replace the second full paragraph on page 45, with the following rewritten paragraph:

The forward primer used for these PCR reactions was designated NMASP-1-Nco (49 mer, forward primer) and NMASP-1-RI (54 mer, reverse primer) and contain sequences complementary to 9 N-terminal and last 9 C-terminal residues, respectively, of the putative NMASP protein. In addition to the NMASP coding sequences, the forward primer was designed to contain a unique NcoI restriction site optimally located upstream of the first Met

residue of the NMASP protein while the reverse primer was designed to contain an EcoRI restriction site immediately downstream of the TAA termination codon. These restriction sites were engineered to allow directional cloning and subsequent expression of the NMASP ORF from the commercially available procaryotic expression vector pTrcHis (InvitroGen™). In order to introduce a correctly positioned NcoI site (CCATGG) at the N-terminus of the ORF, it was necessary to change the first base of the second codon (CTC) from C to G which effects a conservative residue substitution at this position (Leu=>Val).

Please replace the first full paragraph on page 46, with the following rewritten paragraph:

Standard PCR amplification reactions (2 mM Mg.sup.2+, 200 umol dNTPs, 0.75 units AmpliTaq®, 50 ul final volume) were programmed using ~0.0 ug of N. meningitidis B chromosomal DNA. Separate reactions were programmed using DNA from N. meningitidis type B strains H44/76, 250338, and BZ198. Amplification of target sequences was achieved using a standard 32-cycle, three-step thermal profile, i.e. 95° C., 30 sec; 60° C., 45 sec, 72° C., 1 min. Amplification was carried out in 0.2 ml polypropylene thin-walled PCR tubes (Perkin-Elmer) in a Perkin-Elmer model 2400 thermal cycler. All three reactions produced the NMASP-specific ~1.4 Kbp amplimer.

Please replace the second full paragraph on page 46, with the following rewritten paragraph:

The ~1.4 Kbp NMASP amplimer was purified from unincorporated primers using hydroxyapatite spin columns (QiaGen®) and digested to completion with an excess of NcoI and EcoRI (BRL, ~10 units per lug DNA). The purified and digested rNMASP ORF was then purified as described above and cloned into the commercially available expression plasmid pTrcHisB that had been previously digested with both NcoI and EcoRI and treated with calf intestinal phosphatase to prevent vector religation (5:1, insert:vector ratio). Aliquots from the ligation reaction were then used to transform a suitable E. coli host (e.g. TOP10) to ampicillin resistance. Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared using commercially available reagents (QiaGen® Mini Prep Kit) and examined for the presence of recombinant plasmids larger than the ~4.4 Kbp vector plasmid pTrcHis (i.e. insert-carrying plasmids). Large recombinant plasmids were then digested to completion with NcoI and EcoRI and examined for the presence of the ~1.4 Kbp NMASP-specific fragment by standard agarose gel electrophoresis. All ~5.8 Kbp plasmids tested were found to contain the NMASP insert. Plasmid pNmAH116 was one recombinant derivative isolated by these procedures. A map of plasmid pNmAH116 is depicted in FIG. 1.

Please replace the first full paragraph on page 47, with the following rewritten paragraph:

The amplification primers used in these PCR reactions were designated NMASP-G3-F-Nco (42 mer, forward primer) and NMASP-G3-RCf-Xba (47 mer, reverse primer). In addition to the NMASP coding sequence, the forward primer was designed to contain a unique NcoI restriction site optimally located upstream of the Ala_{24} residue. Similarly, the reverse primer was designed to contain an XbaI restriction site immediately downstream of the last NMASP codon (CAA, Q). The 3' XbaI restriction site was engineered into the primer such that the NMASP coding sequence would be fused in frame to a myc antibody detection domain and a C-terminal $(His)_6$ affinity purification tag encoded on the pBAD/gIII (InvitroGen™) vector plasmid.

Please replace second full paragraph on page 47, with the following rewritten paragraph:

Standard PCR amplification reactions (2 mM Mg.sup.2+, 200 umol dNTPs, 0.75 units AmpliTaq®, 50 ul final volume) were programmed using ~0.1 ug of N. meningitidis B H44/76 chromosomal DNA. Amplification of the NMASP target sequence was achieved using a standard 32-cycle, three-step thermal profile, i.e. 95° C., 30 sec; 60° C., 45 sec, 72° C., 1 min. Amplification was carried out in 0.2 ml polypropylene thin-walled PCR tubes (Perkin-Elmer) in a Perkin-Elmer model 2400 thermal cycler. PCR reactions produced the predicted NMASP-specific ~1.3 Kbp amplimer.

Please replace paragraph bridging pages 47 and 48, with the following rewritten paragraph:

The ~1.3 Kbp NMASP PCR product was purified from unincorporated primers using hydroxyapatite spin columns (QiaGen®) and digested to completion with an excess of NcoI and XbaI (BRL, ~10 units per lug DNA) according to the manufacturers recommendations. The purified and digested rNMASP ORF was then purified as described above and cloned into the commercially available expression plasmid pBAD/gIII that had been previously digested to completion with both NcoI and XbaI and treated with calf intestinal alkaline phosphatase (CIAP, BRL, ~0.05 units/pmole 5' ends) to prevent vector religation (~5:1, insert:vector ratio). Aliquots from the ligation reaction were then used to electrotransform a suitable E. coli host (e.g. TOP10, InvitroGen™). Transformed cells were plated on 2X-YT agar plates containing 100 ug/ml ampicillin and cultured for ~12-18 hours at 37° C. Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared using commercially available reagents (QiaGen® Mini Prep Kit) and examined for the presence of recombinant plasmids larger than the ~4.1 Kbp vector plasmid pBAD/gIII (i.e. insert-carrying plasmids). These putative insert-carrying recombinant plasmids were then digested to completion with NcoI and XbaI and examined for the presence of the ~1.3 Kbp NMASP-specific fragment by standard agarose gel electrophoresis (0.8% agarose, TAE buffer). All ~5.4 Kbp plasmids tested were found to contain the NMASP insert. Plasmid pNmAH145 was one recombinant derivative isolated by these procedures.

Please replace the last full paragraph on page 48, with the following rewritten paragraph:

Samples (~1.0 ml) of both induced and non-induced cultures were removed following the induction period and cells collected by centrifugation in a microcentrifuge (13kXg; Eppendorf™) at room temperature for ~3-5 minutes. Individual cell pellets were suspended in ~50ul of sterile water, then mixed with an equal volume of 2XLamelli SDS-PAGE sample buffer containing 2-mercaptoethanol, and placed in boiling water bath for ~3-5min to denature and reduce the recombinant protein. Equal volumes (~15 ul) of both the arabinose-induced and the non-induced cell lysates were loaded onto duplicate 4-20% Tris/glycine polyacrylamide gradient gels (1 mm thick Mini-gels, Novex®). The induced and non-induced lysate samples were electrophoresed together with prestained molecular weight markers (SeeBlue®, Novex®) under conventional electrophoresis conditions (~30 mA, constant current) using a standard SDS/Tris/glycine running buffer (BioRad).

Please replace the paragraph bridging pages 48 and 49, with the following rewritten paragraph:

Following electrophoresis, one gel was stained with commassie brilliant blue R250 (BioRad) and then destained using an acetic acid:methanol:water destaining solution to visualize novel ~50 kDa NMASP arabinose-inducible protein. The second gel was electroblotted onto a PVDF membrane (0.45 micron pore size, Novex®) for ~2hrs at 4°C. and ~125 mA constant current using a BioRad Mini-Protean II blotting apparatus and Towbin's methanol-based (20%) transfer buffer. Blocking of the membrane and antibody incubations were performed using a Tris (50 mM,pH7.3):$CaCl_2$ (1 mM): Tween-20® (0.2%) buffer containing 0.5% casein. A monoclonal anti-$(His)_5$ antibody conjugated to HRP (QiaGen®) was used at a 1/5,000 dilution to confirm the expression and identify of ~50 kDa inducible rNMASP protein. Visualization of the antibody reactive pattern was achieved on Hyperfilm™ using the Amersham ECL chemiluminescence system. The results from this experiment are shown in ~~FIG. 2~~ FIGS. 2A and 2B.

Please replace the last full paragraph on page 49, with the following rewritten paragraph:

The general process for the purification of NMASP protein as a soluble protein is given below. Insoluble material is removed after French press disruption by high speed centrifugation (~10,000 Xg, 4° C., 30min). The soluble fraction containing NMASP is suspended in ~20 ml of ice cold 50 mM Tris-HCl buffer (pH8.0) and loaded onto a DEAE-Sephacel® (Pharmacia) ionic exchange column (~5 cmX60 cm). To minimize autoproteolysis of the NMASP protein, chromatography is conducted at 4° C. Unbound material is washed from the column using loading buffer (50 mM Tris-Hcl, pH8.0) prior to elution of bound NMASP protein. Elution of NMASP from the Sephacel® matrix is achieved using a NaCl gradient (0.05-0.5M NaCl, in 50 mM Tris-Hcl, pH8.0). Fractions released by the salt gradient are collected and examined by standard SDS-gel electrophoresis methodologies for the presence of a ~40-55 kd protein. Fractions are also assayed for protease activity using a standard azocasein colorimetric assay. Fractions containing NMASP are pooled and extensively dialyzed against 10 mM sodium phosphate buffer (SPB, pH8.0) at 4° C.

Please replace the first full paragraph on page 50, with the following rewritten paragraph:

The partially purified NMASP is then applied to a hydroxylapatite column, previously equilibrated in SPB. Bound proteins are eluted using a 0.1-0.5M NaCl gradient in SPB. Fractions are collected periodically during elution and examined for the presence of NMASP by SDS-gel electrophoresis and protease activity as above. Eluted material is dialyzed against 50 mM Tris-HCl to remove residual salt and concentrated using a Centricon-30™ concentrator (Amicon, 30,000 MWCO).

Please replace the second full paragraph on page 50, with the following rewritten paragraph:

The sequence information shown above is used to design a pair of nondegenerate convergent (i.e. one forward and one reverse primer) oligonucleotide NMASP-specific primers. PCR amplification of DNA fragments is performed under the same conditions as described above

with the exception that the annealing temperature is raised to 50° C. The DNA fragment is isolated from an agarose gel as before and radiolabelled using [32P]-gamma-ATP and T4 polynucleotide kinase according to standard methods. Unincorporated radiolabel is separated from the probe on a G25 Sepharose™ spin column. Before use, the probe is denatured for 2 min. at 95° C. and subsequently chilled on ice (4° C.).

Please replace the third full paragraph on page 50, with the following rewritten paragraph:

Phage plaques from library platings are immobilized on nylon filters using standard transfer protocols well known to those skilled in the art. Digested bacterial genomic DNA, phage or plasmid DNA is electrophoresed on 0.8% TAE-agarose gels and transferred onto nylon filters using a pressure blotter (Stratagene®) according to the manufacturer's recommendations. Hybridizations with selected probes are performed at 37° C. Hybridizations with other probes are generally carried out at 60° C. Washes of increasing stringency are done at the respective hybridization temperatures until nonspecific background is minimized.

Please replace the paragraph bridging pages 50 and 51, with the following rewritten paragraph:

A genomic library is constructed in the λZAPII replacement vector obtained from Stratgene. The vector arms are digested with EcoR1. Digests of Neisseria meningitidis DNA by Eco R1 are performed to yield fragment sizes between 1 kb and 5 kb. Ligations of vector arms and insert DNA are carried out according to standard protocols. Ligation reactions are packaged in vitro using the Stratagene® GigaPack Gold III extract. The packaged phage are plated on E. coli X1 Blue MRA (P2) (Stratagene®). An initial library titer is determined and expressed as number of pfu.

Please replace the third full paragraph on page 51, with the following rewritten paragraph:

Sequencing of the NMASP nucleic acid from pNMASP-3 was performed using the plasmid pNMASP as a template. All sequencing reactions were performed using the Dye Terminator Cycle Sequencing Kit from Perkin-Elmer according to the manufacturer's specifications. The sequencing reactions were read using an ABI Prism® 310 Genetic Analyzer. The sequences were aligned using the AutoAssembler™ software (Perkin-Elmer) provided with the ABI Prism® 310 sequencer. This plasmid was inserted into E. coli Top10 (InvitroGen™) and deposited with American Type Culture Collection (ATCC® (American Type Culture Collection)) as E. coli Top10 (pNMAH116).

Please replace the fourth full paragraph on page 53, with the following rewritten paragraph:

E. coli Top10 containing plasmid NMAH116 (pNmAH116), was deposited on Aug. 21, 1998 with the American Type Culture Collection (ATCC® (American Type Culture Collection)). 10801 University Boulevard, Manassas Va., 20110-2209, USA, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession No. 98839.